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Corporate Medical Policy

Genetic Testing for Rett Syndrome AHS – M2088

File Name: genetic_testing_for_rett_syndrome
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Description of Procedure or Service

Description

Rett syndrome (RTT) is a rare X-linked neurodevelopmental disorder that occurs almost exclusively in females and is usually caused by mutations in the methyl CpG binding protein 2 (*MECP2*) gene (Amir et al., 1999). It is characterized by normal early growth and development, followed by regressions in development, walking, language, and purposeful use of the hands, along with slowed brain and head growth, distinctive hand movements, seizures, and intellectual disability (Colvin et al., 2004; Hagberg et al., 1983; Leonard et al., 2017; Naidu et al., 1986; Neul et al., 2010; Rett, 1966).

Terms such as male and female are used when necessary to refer to sex assigned at birth.

******Note: This Medical Policy is complex and technical. For questions concerning the technical language and/or specific clinical indications for its use, please consult your physician.***

Policy

BCBSNC will provide coverage for genetic testing for Rett syndrome when it is determined the medical criteria or reimbursement guidelines below are met.

Benefits Application

This medical policy relates only to the services or supplies described herein. Please refer to the Member's Benefit Booklet for availability of benefits. Member's benefits may vary according to benefit design; therefore member benefit language should be reviewed before applying the terms of this medical policy.

When Genetic Testing for Rett Syndrome is covered

For a child with developmental delay/intellectual disability and signs/symptoms of Rett syndrome (RTT), but for whom there is uncertainty in the clinical diagnosis, confirmation of a diagnosis of RTT using genetic testing for a *MECP2, CDKL5* (Cyclin-Dependent Kinase-Like 5) and/or *FOXG1* (Forkhead Box G1) mutation on the X chromosome is considered medically necessary.

When Genetic Testing for Rett Syndrome is not covered

All other indications for mutation testing for Rett syndrome, including prenatal screening and testing of family members, are not covered.

Policy Guidelines

Background

Rett syndrome (RTT) is a severe neurodevelopmental disorder which affects approximately 1:10,000 live female births in the United States annually (Hagberg, 1985; NORD, 2019). It is a prominent cause of severe intellectual disability in women, accounting for up to 10% of cases inherited genetically (Armstrong, 1997). Originally thought to be lethal in males (Amir et al., 1999; Chahil et al., 2018; Franco & Ballabio, 2006), RTT has been identified in up to 1.3% of male patients with mental retardation (Villard, 2007) and can be associated with a more severe phenotype (Zhang et al., 2017). These males have either an extra X-chromosome (Klinefelter syndrome) or somatic mosaicism of the *MECP2* variant. Reichow et al. (2015) claim to have published the first review of male RTT data in 2015, and they only identified a total of 57 published cases.

Rett syndrome can be inherited as an X-linked dominant disorder; however, more than 99% of cases result from a de novo pathogenic mutation in the *methyl CpG binding protein 2* (*MECP2*) gene (Amir et al., 1999; Christodoulou & Ho, 1993), a transcriptional regulator located on the X chromosome. More than 200 mutations in *MECP2* have been associated with RTT (Suter et al., 2014). Analysis of parental origin of the mutated *MECP2* gene in sporadic cases of RTT showed that 94.4% of mutations were from paternal origin, 90.6% of which were point mutations; further, 5.6% of mutations were from maternal origin (Zhang et al., 2012). This may explain the high occurrence of RTT in females. MECP2 is a multifunctional protein which interprets DNA methylation and regulates chromatin architecture, gene transcription, and RNA splicing (Sun et al., 2018). The complex upstream and downstream pathways of *MECP2* involve microRNAs and neurotrophic factors, such as GABA and BDNF (Kang et al., 2014). Transcriptome level analysis in tissues derived from RTT patients report dysregulations in dendritic connectivity and synapse maturation, mitochondrial dysfunction, and glial cell activity (Shovlin & Tropea, 2018). Researchers have identified two individuals with an RTT diagnosis who lacked a mutation in the *MECP2* gene but had a mutation in other genes previously unassociated with RTT: *CTNNB1* and *WDR45* (Percy et al., 2018).

The *MECP2* gene is critical for neuronal maturation (Fukuda et al., 2005; Smrt et al., 2007), and its deficiency results in impaired dendritic morphogenesis and reduced dendritic spine numbers (Chapleau et al., 2009; Kishi & Macklis, 2010). This results in dysfunctional synaptic transmission and neural network activity (Sun et al., 2018), affecting successive stages of brain development, including prenatal neurogenesis, postnatal development of synaptic connections and function, experience-dependent synaptic plasticity, and maintenance of adult neural function, including sensory integration (Feldman et al., 2016).

The clinical picture of RTT is characterized by a broad clinical spectrum of signs and symptoms (Pini et al., 2016) and a distinctive course of apparent normal development for the first six to 18 months of life, followed by characteristic developmental stagnation and loss of acquired skills, including loss of intellectual functioning, loss of acquired fine and gross motor skills and communication (Colvin et al., 2004; Dolce et al., 2013; Hagberg et al., 1983; Leonard et al., 2017; Naidu et al., 1986; Neul et al., 2010; Rett, 1966). Purposeful use of the hands is often replaced by repetitive stereotypical hand movements (Dy et al., 2017; Elian & de, 1996; Goldman & Temudo, 2012). Other clinical observations include deceleration of head growth, seizures, disturbed breathing patterns, scoliosis, growth retardation, and gait apraxia (Cianfaglione et al., 2015).

Despite a period of apparently normal early development, the profound neurological regressions characteristic of RTT have been found to result from *MECP2*-related defects in the establishment and refinement of early neural circuits and, later, cortical plasticity (Feldman et al., 2016). Subtle signs, such as hypotonia, jerkiness in limb movement, and limited social interaction can be present during early infancy (Ip et al., 2018).

The severity and rate of progression of this disease can vary with several recognized atypical variants. The milder forms (Zappella) present with less severe regression and milder expression of the clinical characteristics of RTT. In the most severe forms, there is no normal development period (Neul et al., 2010). Both genetic and clinical variants of RTT are associated with distinct electrophysiological profiles reflecting how genetic dysregulation of synapse formation results in differences in neuronal network architecture (Sun et al., 2018) and varying clinical phenotypes (Keogh et al., 2018). The pattern of X-chromosome inactivation can also influence the severity of the clinical disease (Archer et al., 2007; Weaving et al., 2003).

Mutations in the upstream cyclin-dependent kinase-like 5 (*CDKL5*) gene cause an early seizure (Hanefield) variant of the RTT phenotype (Bahi-Buisson et al., 2008), and mutations in the forkhead box G1 (*FOXG1*) gene have been found in the congenital variant (Rolando) (Ariani et al., 2008). Two cases of females with pathogenic de novo mutations in *SCN1A*, which usually leads to Dravet syndrome, but fulfill the diagnostic criteria for classic RTT have also been reported (Henriksen et al., 2018). In males, *MECP2* duplication phenotypically presents with infantile hypotonia, recurrent respiratory infections, and severe mental retardation (Villard, 2007).

Fu et al. (2020) published a set of "consensus guidelines" with input from several clinical sites, Rett Syndrome-focused centers, two patient advocacy groups, and Rett Syndrome clinical specialists. Although this guideline focuses on "management" of Rett Syndrome, the guideline does comment on the genetics of Rett Syndrome. The guideline remarks that "nearly" all individuals with Rett Syndrome (RTT) have a loss-of-function mutation on the *MECP2* and that these mutations are "almost always" *de novo* (and thereby not expected to recur in families). Two other genes (*CDKL5* and *FOXG1*) are named as possible causes of RTT. The guideline does not note any specific treatments based on type of mutation, though two other genes (*CDKL5* and *FOXG1*) are named as possible causes of RTT. However, the guideline states that "Alterations in *MECP2*, *CDKL5* and *FOXG1* should be considered in all individuals, male and female, with developmental delays and intellectual disability" (Fu et al., 2020). This consensus guideline also notes there is hope for disease-modifying therapy as reversing symptoms in mice has occurred in a clinical research context (Fu et al., 2020).

Banerjee et al. (2019) published a paper titled "Towards a Better Diagnosis and Treatment of Rett Syndrome: A Model" summarizing the developments in the diagnosis and treatment of Rett syndrome over the past 50 years. They note that the first gene therapy trial for *MECP2* "was modelled after the successful (i.e., improved survival and motor functions) single dose intravenous adeno-associated virus serotype 9 delivery of complementary DNA." Even with promising gene therapy techniques, the authors note that the field has challenges. "The Rett syndrome field is experiencing the same challenges as other neurodevelopmental disorders pursuing neurobiologically based treatments: inadequate outcomes and measures of response." The authors also comment on the complexity of the *MECP2* mutation's role in the syndrome, "*MECP2* mutations is supportive, but not confirmatory because of the limited genotype-phenotype correlations in Rett syndrome" (Banerjee et al., 2019).

Confirmation of the genetic diagnosis can improve the medical management of the patient. It can also end the diagnostic odyssey, provide a general idea of prognosis for the patient, and/or provide closure to the family (Mroch et al., 2012). Complex neurodevelopmental disorders need multi-disciplinary treatment approaches for optimal care. The clinical effectiveness of treatments is limited in patients with rare genetic syndromes and multisystem morbidity such as RTT; single drug strategies may not be sufficient, due to the multiple overlapping physiological systems affected (Singh & Santosh, 2018).

Functional performance for self-care, upper extremity function, and mobility in RTT patients may relate to the type of mutation. Knowledge of these relationships is useful for developing appropriate rehabilitation strategies and prognosis (Pidcock et al., 2016).

Of the clinical criteria for RTT, loss of hand skills was the most significant clinical predictor of a positive genetic test for mutations of a *MECP* gene in females. Gait abnormalities and stereotypic hand movements were also strong predictors of a positive genetic test for mutations of *MECP*. Language delay is the least specific of the major criteria (Knight et al., 2016). A reliable and single multidimensional questionnaire, the Rett Evaluation of Symptoms and Treatments (REST) Questionnaire, is being developed to combine physiological aspects of the disease obtained using wearable sensor technology, along with genetic and psychosocial data to stratify patients and streamline the care pathway (Santosh et al., 2017).

Clinical Utility and Validity

Lallar et al. (2018) used Sanger sequencing to diagnose suspected RTT cases. Participants were divided into two groups: Group 1 was comprised of females with symptoms of classical and atypical RTT, and Group 2 was comprised of females with other "Rett like features" that did not fit into the first category. *MECP2* mutations were identified in 74% of females in Group 1 and in 0% of females in Group 2; females in Group 1 with classical RTT had a mutation detection rate of 93% (Lallar et al., 2018). This shows that Sanger sequencing is efficient in detecting RTT in patients with the classical form of the disease.

Sheinerman et al. (2019) used brain-enriched microRNAs (miRNAs) to identify miRNA biomarkers of RTT; for this study, 30 patients with RTT were matched with 30 healthy controls of similar age (Sheinerman et al., 2019). Results showed that miRNAs identified RTT patients with 85-100% sensitivity when compared to controls; further, the researchers determined that "the dynamics in levels of miRNAs appear to be associated with disease development (involvement of liver, muscle and lipid metabolism in the pathology)" (Sheinerman et al., 2019). These results suggest that circulating miRNAs could be used to measure RTT disease progression or individual response to treatment.

In at least 95% of Rett syndrome cases, the cause is a de novo mutation in the child; *MECP2* variants are rarely inherited from a carrier mother with a germline mutation in *MECP2*, in whom favorable skewing of X-chromosome inactivation results in minimal to no clinical findings. When the mother is a known carrier, inheritance follows an X-linked dominant pattern with a 50% risk to her offspring of inheriting the *MECP2* variant (Christodoulou & Ho, 1993).

A mutation in *MECP2* does not necessarily equate to a clinical diagnosis of RTT. *MECP2* mutations have also been reported in other clinical phenotypes, including individuals with an Angelman-like picture, nonsyndromic X-linked intellectual disability, autism, in males as PPM-X syndrome (an X-linked genetic disorder characterized by psychotic disorders, parkinsonism, and intellectual disability), and most commonly as neonatal encephalopathy (Liyanage & Rastegar, 2014; Suter et al., 2014; Williamson & Christodoulou, 2006).

Recent expert opinion in the United Kingdom concluded that genetic testing for all children with unexplained global developmental delay (GDD) should be first-line if an exogenous cause is not already established. All patients, irrespective of severity of GDD, should have investigations for treatable conditions. The yield for treatable conditions is higher than previously thought and that investigations for these conditions should be considered as first-line. Additional second-line investigations can be led by history, examination, and developmental trajectories (Mithyantha et al., 2017).

Vidal et al. (2017) have utilized next generation sequencing (NGS) in a total of 1577 patients with RTT-like clinical diagnoses or patients with potential RTT genetic mutations as determined previously by Sanger Sequencing. Of the 1577 patients with RTT-like clinical diagnoses, the NGS method was able to confirm the RTT diagnosis in 477 patients (about 30%). Further, "Positive results were found in 30% by Sanger sequencing, 23% with a custom panel, 24% with a commercial panel and 32% with whole exome sequencing," suggesting that NGS is a competitive diagnostic RTT tool compared to the aforementioned methods (Vidal et al., 2017).

Vidal et al. (2019) used multiplex ligation-dependent probe amplification (MLPA) in the *MECP2* gene of 21 RTT patients to identify deletions of varying sizes; these researchers identified both total and partial deletions of the *MECP2* gene in each patient, with identified partial deletions ranging from 1,235 bp to 85 kb. Breakpoints were delineated by DNA-qPCR; the results have allowed the researchers to "propose a genotype–phenotype correlation" which will assist in appropriate genetic counseling (Vidal et al., 2019).

Seventy-two classical Rett syndrome (RTT) female patients were included in a cohort study by Khajuria et al. (2020) to analyze exons 2-4 of *MECP2* gene by Sanger sequencing for sequence variations followed by deletion/duplication analysis using Multiplex Ligation-dependent Probe Amplification (MLPA). Patients were defined as classical when they showed signs of partial or complete loss of acquired purposeful hand skills, partial or complete loss of acquired spoken language, gait abnormalities, impaired or absence of ability to walk, and stereotypic hand movements. Through Sanger Sequencing, *MECP2* sequence variations were identified in 90.3% of patients. With further evaluation using MLPA, large deletions of *MECP2* were identified in 9.7% of the patients, which were negative on DNA sequencing. MLPA analysis increased the detection rate of *MECP2* sequence variants identified in patients from 90.3% to 98.6%. The authors emphasize that "MLPA analysis of *MECP2* is crucial and needs to be performed in classical RTT patients. Large deletions can be missed using DNA sequencing and reaffirms the view that large *MECP2* deletions are an important cause of classical RTT (Khajuria et al., 2020)." Xiol et al. (2021) performed a clinical review of technological advances in RTT genetics. The authors review summarizes that our understanding of Rett syndrome has evolved "towards a spectrum of overlapping phenotypes with great genetic heterogeneity." The authors note that advances in genetic diagnosis have been impacted by the rise in next generation sequencing (NGS) and whole genome sequencing. Of note are the "90 causative genes" and "significantly overlapping phenotypes" involved in RTT spectrum disorders. To achieve an accurate and quick diagnosis of Rett syndrome, the authors strongly recommend simultaneous multiple gene testing and thorough phenotypic characterization. Bassuk (2021) published a paper concerning methyl-CpG-binding protein 2 (*MECP2*) and its encoding of an epigenetic reader, MeCP2. The author notes that loss of function of the epigenetic reader may be a factor in RTT, but also that "locus duplications also cause a severe neurodevelopmental disorder, *MECP2* duplication syndrome (MDS)." This suggests that MeCP2 (the protein) could be what is called a "Goldilocks protein," that is, one that requires an activity level that is precise. Using the re-expression of the MeCP2 protein in mouse models, the author presents a case for the development of therapeutic interventions in people and the restoration of the desirable phenotypes. However, gene therapy must be approached with caution, as restoring function to the protein still carries the risk of "MDS overexpression phenotypes" (Bassuk, 2021).

Guidelines and Recommendations

American Academy of Pediatrics (AAP)

A 2014 policy statement from the AAP recommends *MECP2* mutation analysis for females with microcephaly or deceleration of head growth and other features of Rett syndrome, or who present with stereotypical hand-wringing movements and developmental regression. *MECP2* gene mutations are extremely rare in males but may be considered in boys who present with clinical features of Rett syndrome or severe developmental regression (Moeschler & Shevell, 2014).

Complete *MECP2* deletion, duplication, and sequencing study is also recommended for females with intellectual disability or global developmental delay for whom the chromosomal microarray, specific metabolic testing, and fragile X genetic testing did not produce a diagnosis (Moeschler & Shevell, 2014).

The above guideline was reaffirmed in 2019 (AAP, 2019).

The AAP also published a guideline focusing on children with autism spectrum disorder (ASD). In it, they note that other disorders may meet certain criteria for ASD. However, the AAP notes that these disorders should prompt the "appropriate targeted testing" (or referral to a specialist). The AAP lists an example of Rett Syndrome, stating that "for example, a girl with significant developmental delays, deceleration in head growth velocity, and characteristic midline hand movements should prompt genetic testing for a mutation or deletion or duplication of *MECP2*, the gene implicated in Rett syndrome." In Supplemental Table 13, they list the following findings as representative of Rett Syndrome: "Deceleration of head growth velocity, acquired microcephaly, loss of purposeful hand use, prominent hand stereotypies (especially hand wringing or clasping), apraxia, hyperventilation or breath-holding, seizures" (Hyman et al., 2020).

Canadian Pediatric Society (CPS)

The CPS supports the guidelines mentioned above by the AAP. The CPS stated that "According to the AAP. . . *MECP2* molecular analysis should be ordered when characteristic symptomatology is present (i.e., initially normal development followed by loss of speech and purposeful hand use, stereotypical hand movement, gait abnormalities) or for moderately-to-severely affected girls" (Belanger & Caron, 2018).

RettSearch

The AAP has not provided recommendations on when to use *CDKL5* or *FOXG1* testing. RettSearch members, representing the majority of the international clinical RTT specialists, "participated in an iterative process to come to a consensus on a revised and simplified clinical diagnostic criteria for [RTT]" (Neul et al., 2010). This group provided clarifications for diagnosis of classic or typical RTT and atypical RTT and provided guidelines for molecular evaluation of specific variant forms of RTT. The authors define RTT as a clinical diagnosis based on distinct clinical criteria, independent of molecular findings. Presence of a *MECP2* mutation is not sufficient for the diagnosis of RTT. Neul et al. (2010) proposed three distinct criteria for diagnosis of variant forms of RTT: preserved speech variant (Zapella variant), early seizure variant (Hanefeld variant) and congenital variant (Rolando variant); identifying the molecular genetics of each variant was also recommended. In the Zapella variant, the molecular analysis for *MECP2* was recommended. In Hanefeld and Rolando variants, recommended mutations for analysis were in the *CDKL5* and *FOXG1* genes, respectively. Further, it was stated that patients found negative for *MECP2* mutations and who have a strong clinical diagnosis of RTT should be considered for further screening for the *CDKL5* gene if early onset seizures or *FOXG1* gene congenital features (e.g., severe postnatal microcephaly) are present (Neul et al., 2010).

American College of Medical Genetics (ACMG)

In 2013, the ACMG revised its evidence-based guidelines for clinical genetics evaluation of autism spectrum disorders. Testing for *MECP2* mutations is recommended as part of the diagnostic workup of females who present with an autistic phenotype. Routine *MECP2* testing in males with autistic spectrum disorders is not recommended. However, when features of *MECP2* duplications (e.g., drooling, recurrent respiratory infections, hypotonic facies) are present, *MECP2* duplication testing in boys with autism and such features may be considered (Schaefer & Mendelsohn, 2013).

Applicable State and Federal Regulations

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of

1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

Billing/Coding/Physician Documentation Information

This policy may apply to the following codes. Inclusion of a code in this section does not guarantee that it will be reimbursed. For further information on reimbursement guidelines, please see Administrative Policies on the Blue Cross Blue Shield of North Carolina web site at www.bcbsnc.com. They are listed in the Category Search on the Medical Policy search page.

Applicable service codes: 81302, 81303, 81304, 81404, 81405, 81406, 0234U

BCBSNC may request medical records for determination of medical necessity. When medical records are requested, letters of support and/or explanation are often useful, but are not sufficient documentation unless all specific information needed to make a medical necessity determination is included.

Scientific Background and Reference Sources

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Specialty Matched Consultant Advisory Panel review 3/2020

Medical Director review 3/2020

Specialty Matched Consultant Advisory Panel review 3/2021

Medical Director review 3/2021

Medical Director review 1/2022

Medical Director review 1/2023

Policy Implementation/Update Information

1/1/2019 BCBSNC will provide coverage for genetic testing for Rett syndrome when it is determined to be medically necessary because criteria and guidelines are met. Medical Director review 1/1/2019. Policy noticed 1/1/2019 for effective date 4/1/2019. (jd)

4/1/2019 Description section, policy guidelines and references updated. Medical Director review 4/2019. (jd)

10/29/19 Wording in the Policy, When Covered, and/or Not Covered section(s) changed from Medical Necessity to Reimbursement language, where needed. (hb)

2/11/20 Annual review by Avalon 4th Quarter 2019 CAB. No revisions and no change to policy intent. Medical Director review 12/2019. (jd)

3/31/20 Specialty Matched Consultant Advisory Panel review 3/2020. Medical Director review 3/2020. (jd)

2/9/21 Annual review by Avalon 4th Quarter 2020 CAB. Related Policies added to description section. Policy guidelines and references updated. Added PLA code 0234U to the Billing/Coding section, effective 4/1/21. Medical Director review 1/2021. (jd)

3/31/21 Specialty Matched Consultant Advisory Panel review 3/2021. Medical Director review 3/2021. (jd)

2/8/22 Reviewed by Avalon 4th Quarter 2021 CAB. Acronyms for CDKL5 and FOXG1 spelled out in item #1 under the When Covered section for clarity. No change to policy intent. Description, policy guidelines, and references updated with minor revisions. Medical Director review 1/2022. (jd)

2/7/23 Reviewed by Avalon 4th Quarter 2022 CAB. Description, Policy Guidelines and References sections updated, Related Policies removed. When Covered section edited for clarity, no change to policy statement. Medical Director review 1/2023. (tm)